Acute Exertion Elicits a $H_2O_2$-Dependent Vasodilator Mechanism in the Microvasculature of Exercise-Trained but not Sedentary Adults

Matthew J. Durand, Kodlipet Dharmashankar, Jing-Tan Bian, Emon Das, Mladen Vidovich, David D. Gutterman, Shane A. Phillips

Abstract—Brachial artery flow–mediated vasodilation in exercise-trained (ET) individuals is maintained after a single bout of heavy resistance exercise compared with sedentary individuals. The purpose of this study was to determine whether vasodilation is also maintained in the microcirculation of ET individuals. A total of 51 sedentary and ET individuals underwent gluteal subcutaneous fat biopsy before and after performing a single bout of leg press exercise. Adipose arterioles were cannulated in an organ bath, and vasodilation to acetylcholine was assessed—the endothelial nitric oxide inhibitor L-NG-nitroarginine methyl ester, the cyclooxygenase inhibitor indomethacin, or the hydrogen peroxide scavenger polyethylene glycol catalase. Separate vessels (isolated from the same groups) were exposed to an intraluminal pressure of 150 mm Hg for 30 minutes to mimic the pressor response, which occurs with isometric exercise. Vasodilation to acetylcholine was reduced in microvessels from sedentary subjects after either a single weight lifting session or exposure to increased intraluminal pressure, whereas microvessels from ET individuals maintained acetylcholine-mediated vasodilation. Before weight lifting, vasodilation of microvessels from ET individuals was reduced in the presence of L-NG-nitroarginine methyl ester and indomethacin. After weight lifting or exposure to increased intraluminal pressure, polyethylene glycol catalase significantly reduced vasodilation, whereas L-NG-nitroarginine methyl ester and indomethacin had no effect. These results indicate that (1) endothelium-dependent vasodilation in the microvasculature is maintained after heavy resistance exercise in ET individuals but not in sedentary subjects and that (2) high pressure alone or during weight lifting may induce a mechanistic switch in the microvasculature to favor hydrogen peroxide as the vasoactive mediator of dilation. (Hypertension. 2015;65:00-00.) • Online Data Supplement

Key Words: hydrogen peroxide ● hypertension ● nitric oxide ● reactive oxygen species ● vasodilation

Aerobic exercise promotes a multitude of health benefits, including improving cardiorespiratory fitness and vascular reactivity of large and small arteries. The effects of resistance exercise on cardiovascular function are less well understood, and recent evidence suggests that a single bout of heavy resistance exercise causes prolonged endothelial dysfunction in the conduit arteries of sedentary subjects who do not regularly exercise. This is in stark contrast to exercise-trained (ET) individuals, as their large artery endothelial function is maintained after partaking in an identical session of heavy resistance exercise. One question that remains unanswered is whether the differential effects of heavy resistance exercise between sedentary and ET subjects extend to arterioles that are responsible for regulating tissue perfusion. Therefore, in this study, we tested the hypothesis that adults who regularly engaged in exercise training are protected from microvascular dysfunction that occurs immediately after heavy resistance exercise compared with sedentary subjects.

A primary difference in the cardiovascular response to aerobic and isometric exercise is the strong pressor response that occurs during heavy isometric exercise. Systolic blood pressures $>$400 mm Hg have been reported in elite weight lifters performing leg press exercise. Another question that remains unanswered is whether the difference in the vascular response to weight lifting (WL) between sedentary and ET subjects is because of the effects of high intraluminal pressure on the vascular wall during this pressor response and whether the microvasculature of ET subjects is adapted to function normally after high pressure stress. Therefore, we also tested the hypothesis that the different vascular response to WL between ET and sedentary subjects could be recapitulated by exposing microvessels to increased intraluminal pressure in the laboratory in the absence of exercise-induced changes in circulating neurohumoral factors that occurs in vivo.
Methods

Subject Population and Recruitment

All protocols were approved by the Institutional Review Boards at the Medical College of Wisconsin and the University of Illinois at Chicago. A total of 54 men and women between the ages of 18 and 40 years were enrolled in this study. Thirty-three subjects were ET individuals who participated in ≥3 WL sessions per week or ran ≥215 miles per week ≥26 months before study, whereas the other 21 participants did not regularly exercise. Based on previous findings which indicate that brachial artery flow-mediated dilation (FMD) is similarly protected in weight lifters, runners, and cross-trainers after a bout of heavy resistance exercise, ET subjects were pooled together into 1 group (7 runners, 9 weight lifters, and 17 cross-trainers). Subjects were excluded if they had known anorexia or bulimia, hypertension, diabetes mellitus, cancer, kidney or liver disease, a blood clotting disorder, anemia, total cholesterol ≥200 mg/dL, used tobacco in the past 6 months, or were currently abusing alcohol or drugs. Female volunteers were excluded if they were pregnant or nursing at the time of the study.

Subjects were recruited via flyers at local health clubs and universities. Once eligibility was confirmed through phone screening, volunteers issued written informed consent and underwent a physical examination to confirm eligibility. Subjects returned for subsequent visits to the study site after fasting for 12 hours and abstaining from exercise for 24 hours to have FMD of the brachial artery assessed via ultrasound or to have a gluteal biopsy procedure performed (see below).

WL and Brachial Artery FMD Protocol

A leg bench press with free weights was used to exercise study participants to maximal exertion, as previously described. All volunteers completed a minimum of 4 sets of 10 repetitions or performed to failure on the final set. FMD and nitroglycerin-mediated dilation were used to measure endothelium-dependent and -independent vasodilation of the brachial artery, respectively, before and 30 minutes after WL using standard techniques.

Gluteal Biopsy Procedure

A gluteal biopsy procedure was either performed with subjects at rest or ≥30 minutes after a subject had performed the isometric WL protocol to maximal exertion, as previously described. Briefly, the skin over the gluteal area was locally anesthetized using lidocaine (2%) and an incision (0.5–1 cm) was made under sterile conditions to expose subcutaneous fat that extrudes through the incision site. The sample was immediately placed in cold HEPES buffer after removal.

Isolated Vessel Protocol and Assessment of Vascular Reactivity

The protocol for assessing agonist-induced vasodilation in isolated human adipose arterioles has been described previously and in detail in the online-only Data Supplement. Vasodilation to acetylcholine (10−6 to 10−4 mol/L) was assessed before and after either WL or a transient increase in intraluminal pressure (60–150 mm Hg for 30 minutes). To examine the role of endothelial nitric oxide synthase (eNOS)-derived NO, cyclooxygenase-derived prostanoids, or the endothelial-derived hyperpolarizing factor H2O2 to acetylcholine-mediated vasodilation, separate vessels were incubated with the eNOS inhibitor L-NG-nitroarginine methyl ester (L-NAME; 10−3 mol/L), the cyclooxygenase inhibitor indomethacin (10−4 mol/L), or the specific H2O2 scavenger polyethylene glycol catalase (PEG-catalase; 500 U/mL), respectively. To assess endothelium-independent dilator capacity, separate vessels were treated with cumulative doses of papaverine (10−6 to 10−4 mol/L).

Statistical Analysis

All data are presented as mean±standard error. Differences between the subjects’ characteristics, brachial FMD and nitroglycerin responses, baseline microvessel diameter, and percentage constriction were compared using an unpaired Student t test. Dilator responses are expressed as percentage dilation, with the maximal diameter of the vessel being equal to 100% dilation. Differences in the arteriolar vasodilator responses to acetylcholine were assessed using a 2-way ANOVA with experimental condition and acetylcholine concentration as parameters. P<0.05 was considered statistically significant.

Results

Baseline subject characteristics for all ET (n=33) and sedentary subjects (n=21) are presented in Table 1. ET individuals were taller and had a lower BMI than sedentary subjects. As expected, ET individuals were able to lift a greater amount of weight on the leg bench press; however, the maximum blood pressure achieved between the groups was similar. The average internal diameter of the adipose microvessels obtained from ET (n=76 vessels) and sedentary (n=40 vessels) subjects was similar (129±7 μm versus 132±10 μm, respectively). Microvessels from each group were constricted to 45±1.9% and 45±2.3% of their maximum diameter, respectively. No differences in cardiometabolic measures or microvascular responses were observed when ET subjects were categorized according to the mode of exercise they participated in (ie, as runners, weight lifters, or cross-trainers; ANOVA; P>0.05; data not shown).

Effect of WL and Increased Intraluminal Pressure on Acetylcholine-Mediated Vasodilation

Consistent with previous studies, FMD of the brachial artery was reduced in sedentary subjects after a single set of leg bench press exercise to maximal exertion (Table 2). Vasodilation to acetylcholine was also significantly reduced in subcutaneous adipose arterioles from the same subjects (Figure 1A). This reduction in dilation acetylcholine was recapitulated by exposing arterioles to increased intraluminal pressure (Figure 1A), indicating that increased pressure on the vessel wall is sufficient to reduce endothelium-dependent vasodilation in healthy, sedentary subjects. Conversely, arterioles from ET subjects maintained acetylcholine-mediated vasodilation after either leg bench press exercise or exposure to increased intraluminal pressure (Figure 1B). The brachial FMD response from the same subjects was not reduced after WL in ET individuals and interestingly showed an increased response compared with baseline (Table 2).

Before WL, combined L-NAME and indomethacin treatment significantly reduced dilation to acetylcholine in microvessels from both sedentary (86% reduction from control) and ET subjects (40% reduction from control), whereas PEG catalase had no effect (Figure 1C and 1D, respectively). These results indicate that under basal conditions acetylcholine-mediated vasodilation is largely mediated by NO/prostanoids in sedentary subjects. In ET subjects, NO/prostanoids also contribute significantly to acetylcholine-mediated dilation, however, to a lesser extent than in sedentary subjects. These data suggest that an unknown endothelial-derived hyperpolarizing factor may contribute to vasodilation in ET subjects under basal conditions. Because PEG-catalase has no effect on vasodilation under control conditions in ET subjects, the endothelial-derived hyperpolarizing factor is not H2O2.

WL had no effect on maximum dilation (sedentary: 84±7.1% pre-WL versus 95±2.2% post-WL; ET: 91±5.8% pre-WL versus 90±1.9% post-WL) or sensitivity (∼log EC50, sedentary: 5.4±0.31 pre-WL versus 5.9±0.31 post-WL; ET: 6.1±0.24 pre-WL versus 5.5±0.17 post-WL) of microvessels.
Table 1. Cardiometabolic Characteristics of Study Participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SED (n=21)</th>
<th>ET (n=33)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male</td>
<td>8</td>
<td>23</td>
<td>NA</td>
</tr>
<tr>
<td>Age</td>
<td>27±0.9</td>
<td>25±1.1</td>
<td>0.202</td>
</tr>
<tr>
<td>Height, cm</td>
<td>169±2.3</td>
<td>176±1.6*</td>
<td>0.013</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>72±3.0</td>
<td>76±2.5</td>
<td>0.314</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25±0.8</td>
<td>24±0.6</td>
<td>0.609</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>27±1.8</td>
<td>17±1.5*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>79±2.4</td>
<td>80±1.7</td>
<td>0.833</td>
</tr>
<tr>
<td>Hip circumference, cm</td>
<td>101±2.2</td>
<td>99±1.7</td>
<td>0.489</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.79±0.02</td>
<td>0.80±0.01</td>
<td>0.477</td>
</tr>
<tr>
<td>Blood glucose, mg/dL</td>
<td>85±2.3</td>
<td>82±2.0</td>
<td>0.412</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>42±0.7</td>
<td>43±0.6</td>
<td>0.104</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>145±11</td>
<td>158±27</td>
<td>0.865</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>106±27</td>
<td>101±6</td>
<td>0.858</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>44±5</td>
<td>49±4</td>
<td>0.442</td>
</tr>
<tr>
<td>Resting SBP, mm Hg</td>
<td>113±1.8</td>
<td>115±2.3</td>
<td>0.412</td>
</tr>
<tr>
<td>Resting DBP, mm Hg</td>
<td>72±1.6</td>
<td>72±1.9</td>
<td>0.939</td>
</tr>
<tr>
<td>Resting heart rate, bpm</td>
<td>65±2.0</td>
<td>67±2.2</td>
<td>0.599</td>
</tr>
<tr>
<td>Maximum SBP, mm Hg</td>
<td>187±6.4</td>
<td>190±5.3</td>
<td>0.728</td>
</tr>
<tr>
<td>Maximum DBP, mm Hg</td>
<td>97±5.8</td>
<td>103±5.5</td>
<td>0.435</td>
</tr>
<tr>
<td>Maximum weight lifted, lb</td>
<td>180±19.6</td>
<td>253±25.2*</td>
<td>0.028</td>
</tr>
</tbody>
</table>

All values are expressed as mean±SEM. DBP indicates diastolic blood pressure; n, number of participants; ET, exercise-trained subjects; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; and SED, sedentary subjects.

*Significant difference (P<0.05) ET vs SED. Cholesterol levels were only obtained for 14 of 21 SED and 14 of 33 ET subjects.

Role of eNOS and Cyclooxygenase in Acetylcholine-Mediated Vasodilation

Inhibition of both eNOS with L-NAME and cyclooxygenase with indomethacin significantly reduced acetylcholine-mediated vasodilation in microvessels obtained from ET subjects before WL (Figure 2). In exact contrast, after a single WL session, L-NAME and indomethacin treatment had no effect on vasodilation (Figure 2). Microvessels exposed to increased intraluminal pressure also maintained dilation in the presence of L-NAME and indomethacin, indicating that neither eNOS nor cyclooxygenase products significantly contribute to the vasodilator response to acetylcholine after a WL session or exposure to transient high pressure stress.

Role of Reactive Oxygen Species in Acetylcholine-Mediated Vasodilation

Treatment with the H₂O₂ scavenger PEG-catalase had no effect on the vasodilator response to acetylcholine in adipose arterioles obtained from ET subjects before WL (Figure 3). After either WL or transient exposure to high pressure, dilation to acetylcholine was reduced in the presence of PEG-catalase, suggesting that heavy resistance exercise contributes to a mechanistic switch in the human microcirculation which favors reactive oxygen species (ROS)-dependent rather than an NO/prostaglandin-dependent vasodilation in ET subjects. This response can be recapitulated by acutely exposing microvessels from ET subjects to high pressure stress, suggesting the hypertensive response to WL may trigger this mechanistic switch.

Discussion

The novel findings in this study are 3-fold. First, endothelial dysfunction that occurs in conduit arteries of sedentary subjects after heavy resistance exercise extends to the microvasculature. Second, acetylcholine-mediated vasodilation is maintained in the microvasculature after a single bout of heavy resistance exercise in ET individuals; however, H₂O₂ (rather than NO/prostaglandins) emerges as the primary vasoactive mediator of dilation. Third, these observed changes in vascular signaling can be recapitulated by transiently increasing intraluminal pressure in isolated cannulated arterioles. These findings suggest that increased intraluminal pressure is the stimulus that blunts vasodilation in sedentary subjects after WL, and it may be the trigger for the observed mechanistic switch in the vasodilator mechanism that occurs within the microvasculature of individuals performing exercise programs to maximal exertion. To our knowledge, this study is the first to demonstrate a mechanistic switch in the peripheral microvasculature to favor ROS-mediated vasodilation in healthy adults.

The effects of increased intraluminal pressure and transient hypertension on microvascular function have been studied in both animal models and in isolated human arterioles, but this is the first study, to our knowledge, to demonstrate a differential response to a pressure stimulus in the microvasculature of ET and sedentary adults. Because WL causes transient increases in blood pressure, it could be hypothesized that this repeated exposure to elevated blood pressures has a conditioning effect on the vasculature. Gündüz and colleagues demonstrated normal acetylcholine-mediated dilation and FMD in mesenteric arteries of spontaneously hypertensive rats after the arteries were exposed to high intraluminal pressure, whereas arteries from normotensive Wistar-Kyoto rats showed blunted dilation to both stimulus after exposure to the same high pressure insult.

Maintaining vasodilation in the microcirculation after exercise is a beneficial adaptation of resistance exercise training, and our data clearly indicate that the microvasculature of ET subjects is highly plastic, as the vasoactive mediator of dilation switches to favor ROS-mediated dilation after hypertensive stress. Under basal conditions, L-NAME and indomethacin caused complete inhibition of acetylcholine-mediated dilation in sedentary subjects; however, dilation was only reduced by ≈40% in ET subjects (Figure 1C and 1D). Thus, it could be surmised that under basal conditions, a yet-identified endothelial-derived hyperpolarizing factor pathway also exists in ET but not in sedentary subjects. Future investigation is warranted to identify this pathway and determine its importance in maintaining vasodilation during stress.
Table 2. Vascular Characteristics of Brachial Artery Before and After Weight Lifting

<table>
<thead>
<tr>
<th>Condition</th>
<th>SED (n=14)</th>
<th>ET (n=14)</th>
<th>Between Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting artery diameter, mm</td>
<td>Pre-WL</td>
<td>Post-WL</td>
<td>Pre-WL</td>
</tr>
<tr>
<td></td>
<td>5.06±0.17</td>
<td>4.99±0.18</td>
<td>5.52±0.27</td>
</tr>
<tr>
<td>Diameter after cuff release, mm</td>
<td>Pre-WL</td>
<td>Post-WL</td>
<td>Pre-WL</td>
</tr>
<tr>
<td></td>
<td>5.47±0.19</td>
<td>5.23±0.17</td>
<td>5.84±0.28</td>
</tr>
<tr>
<td>FMD, %</td>
<td>Pre-WL</td>
<td>Post-WL</td>
<td>Pre-WL</td>
</tr>
<tr>
<td></td>
<td>8.12±1.20</td>
<td>5.13±1.03*</td>
<td>7.31±1.07</td>
</tr>
<tr>
<td>Diameter after NTG, mm</td>
<td>Pre-WL</td>
<td>Post-WL</td>
<td>Pre-WL</td>
</tr>
<tr>
<td></td>
<td>NA</td>
<td>5.99±0.20</td>
<td>NA</td>
</tr>
<tr>
<td>NTG dilation, %</td>
<td>Pre-WL</td>
<td>Post-WL</td>
<td>Pre-WL</td>
</tr>
<tr>
<td></td>
<td>NA</td>
<td>21.74±1.13</td>
<td>NA</td>
</tr>
</tbody>
</table>

All values are expressed as mean±SEM. ET indicates exercise-trained subjects; FMD, flow-mediated dilation; n, number of participants; NTG, nitroglycerin; SED, sedentary subjects; and WL, weight lifting.

*Significant difference (P<0.05) before WL vs after WL.
†Significant difference (P<0.05) ET vs SED subjects.

The switch from NO/prostanoids to a ROS-dependent mechanism of dilation brings into question the physiological cost of maintaining this response. The human vasculature is well adapted to invoke compensatory vasodilator pathways in response to disease or stress. It has been shown that the vasoactive mediator of dilation in both the human heart and the peripheral circulation switches from NO to mitochondrial-derived H$_2$O$_2$ with the onset of coronary artery disease. Conversely, H$_2$O$_2$ has been shown to induce both inflammation and proliferation in vascular cells. Given the opposing effects of NO and H$_2$O$_2$, the physiological consequences of switching the vasodilator mechanism to favor ROS-mediated vasodilation remains to be determined. It could be surmised that in the presence of cardiovascular disease, compensatory H$_2$O$_2$-mediated vasodilation may serve as a feed-forward mechanism that further causes vascular damage in the absence of adequate scavenging. However, other studies have found that H$_2$O$_2$ may...
serve as the stimulus for exercise-induced increases in eNOS expression\textsuperscript{20} and NO generation in the circulation.\textsuperscript{21} The physiological versus pathological ramifications of invoking H\textsubscript{2}O\textsubscript{2}-mediated vasodilation in young, healthy subjects is unclear.

**Study Limitations**

Differences in vascular structure were not evaluated between the sedentary and ET subjects. Greene et al\textsuperscript{22} have used the term athletes artery to describe changes that occur in wall structure, resistance vessel size, and vasodilator function in conditioned athletes. It should be noted that both baseline vasodilator function (Figure 1E) and maximal vessel diameter (Results) were similar between the arterioles from ET and sedentary individuals (Figure 1E); however, vascular wall thickness or differences in wall-to-lumen ratio were not directly measured between the groups. It is possible that repeated exposure to high intraluminal pressures (or increased shear stress) during exercise contributes to the differential vascular response to maximal exertion between the groups through remodeling of the vascular wall, which allows for the athlete microvasculature to better withstand high systolic blood pressure.

Because of small size and the limited number of vessels available from each biopsy specimen, pro- and antioxidant enzyme expression and activity were not measured. Future studies should be conducted specifically to harvest sufficient vessels for immunohistochemical and single vessel polymerase chain reaction analysis of pro- and antioxidant enzymes.

Vessel size also precluded direct measurement of H\textsubscript{2}O\textsubscript{2} release in response to acetylcholine. However, PEG-catalase is highly specific to H\textsubscript{2}O\textsubscript{2}; thus, it is reasonable to conclude that the PEG-catalase–inhibitable component of dilation reflects a direct vasodilator action of H\textsubscript{2}O\textsubscript{2}.\textsuperscript{16,23}

This study raises intriguing questions regarding the specific modality (running, WL, cross-training), intensity, and duration of exercise that is required to cause the vascular adaptations reported here. Future studies designed specifically to address these questions are needed and should provide further insight into the specific adaptations that occur which favor ROS-mediated dilation in the athlete microvasculature.

**Perspectives**

The compensatory ROS-mediated vasodilator pathway that is induced in the presence of coronary artery disease in humans\textsuperscript{11-15} likely occurs over years or decades; however, this study demonstrates activation of a similar pathway after a brief bout of exercise in the microvasculature of athletic individuals. It is intriguing to speculate why athletes would invoke a disease compensatory pathway for vasodilation. Perhaps, maintenance of vasodilation after stress (seen in athletes but not sedentary subjects) is more important than the mechanism of maintaining that dilation, at least in the short-term. Alternatively, the temporary exposure to heightened levels of H\textsubscript{2}O\textsubscript{2} could induce a preconditioning effect, which could elevate NOS and antioxidants to protect against subsequent more stressful vasocompromising stimuli. Exercise should still be considered a beneficial activity because the athlete maintains microvascular vasodilation during stress, whereas the sedentary person does not.

Determining the triggering mechanism to evoke compensatory H\textsubscript{2}O\textsubscript{2}-mediated vasodilation so rapidly in ET subjects could provide therapeutic insights for targeting its chronic induction during cardiovascular disease. Ceramide, a bioactive sphingolipid involved in many cardiovascular disease processes, causes a mechanistic switch in vasodilation from NO to mitochondrial-derived H\textsubscript{2}O\textsubscript{2} in healthy human arterioles.\textsuperscript{24} Inhibition of ceramide production can reverse the disease phenotype and restore NO as the mechanism of FMD in patients with coronary artery disease. It has been also shown...
that the local renin-angiotensin system contributes to pressure-induced endothelial dysfunction in human microvessels in a ROS-dependent manner.1 Future studies should also examine the protective effects of antioxidant therapy or RAS blockade on vascular dysfunction that occurs in response to transient increases in blood pressure.

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Disclosures
None.

References

Novelty and Significance

What Is New?

• Heavy resistance exercise reduces vasodilation in arterioles from sedentary adults but not in conditioned athletes.
• Arterioles from athletes dilate via reactive oxygen species (ROS) rather than NO/prostaglandins after weight lifting.
• The switch in vasodilator mechanism to favor ROS-mediated dilation in microvessels from exercise-trained adults and the reduction in dilation of vessels from sedentary adults can be recapitulated in the laboratory by exposing the isolated arterioles to high intraluminal pressure.

What Is Relevant?

• This study is the first to show induction of a compensatory, ROS-mediat-
ed vasodilator pathway in young, healthy human adults.
• ROS-mediated vasodilation is observed in patients with coronary artery disease, and the same pathway may be induced in athletes after transient hypertension associated with resistance exercise.

Summary
Vasodilation is reduced in arterioles from sedentary subjects after heavy resistance exercise or exposure to increased intraluminal pressure in the laboratory, while arterioles from conditioned athletes maintain dilation, but the mechanism shifts from NO/prostaglandins to ROS.
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Supplementary Material

Acute Exertion Elicits a H₂O₂ – Dependent Vasodilator Mechanism in the Microvasculature of Exercise Trained but not Sedentary Adults

Short Title: Resistance Exercise and Microvascular Function

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Methods:

Flow Mediated Dilation of the Brachial Artery. In the supine state, ultrasound images (GE Logiq 500 pro series) of the brachial artery were visualized in a longitudinal plane at a site one to three centimeters proximal to the antecubital fossa of the arm. Baseline images were recorded and a blood pressure cuff was placed on the forearm and inflated to 200 mmHg for 5 minutes. Arterial diameter was determined during peak hyperemia after release of the blood pressure cuff from the forearm. To assess dilation, 10 seconds of images were captured one, two, and three minutes after cuff release. Flow velocity was recorded at baseline and just after cuff release where maximal velocity was observed. Brachial artery FMD was assessed before and 30 minutes following a single bout of leg press exercise. The original position of the ultrasound probe was marked and measured according to the distance from the antecubital crease and the post-exercise examination was performed in the same position. Ten minutes after recording the last brachial artery diameter measurement following exercise, endothelium-independent dilation was determined with 0.4 mg of sublingual nitroglycerin (NTG) for 5 minutes. Images were recorded and transferred for offline analysis of FMD and NTG responses using edge-detection software (Medical Imaging; Iowa City, IA). The coefficient of variation for our studies was 6.3% for FMD and 3.2% for NTG-induced dilation as previously reported.

General Isolated Vessel Protocol: Adipose tissue freshly removed from the biopsy site was transferred immediately to the laboratory where arterioles were dissected from the tissue. Isolated arterioles were then cannulated onto glass micropipettes of equal diameter at an intraluminal pressure of 20 mmHg in an organ chamber filled with cold physiological salt solution containing (in mmol/L): 123 NaCl, 4.4 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 20 NaHCO₃, 1.2 KH₂PO₄, and 11 glucose. After cannulation, the physiological salt solution was heated to 37°C and bubbled with a 21% O₂-5% CO₂-74% N₂ gas mixture to maintain pH constant at 7.4. After 30 minutes, the intraluminal pressure of the vessel was raised to 60 mmHg for another 30 minute equilibration. The internal diameter of the vessel was then measured using an inverted microscope equipped with a 20X objective, video camera, digital micrometer and video monitor. Human adipose arterioles do not develop intrinsic tone, thus additive 1 µl doses of an endothelin-1 stock solution (10⁻⁸ mol/L) were added to the tissue bath to achieve approximately 50% constriction of the vessels. Vessels that did achieve less than or equal to 25% constriction were excluded from the study.

Assessment of Vasodilator Reactivity: After stable constriction was achieved (no diameter change for 5 minutes), acetylcholine (ACh; 10⁻⁹ – 10⁻⁴ mol/L) was cumulatively added to the organ chamber and the vessel diameter was measured at 1 and 5 minutes after each dose. In separate groups of vessels, the response to graded doses of Ach was assessed before and after a transient increase in intraluminal pressure (60 to 150 mmHg for 30 minutes) followed by 30 minute recovery.

After washing, vessels were constricted a second time with endothelin-1 followed by graded doses of ACh. The percent dilation to ACh or papaverine was calculated using the following equation: \((D_m - D_c)/(D_{max} - D_c) \times 100\) where \(D_m\) is the measured
diameter of the vessel, $D_c$ is the initial constricted diameter and $D_{max}$ is the maximum diameter of the vessel (usually after treatment with the maximal dilator doses of papaverine).

**Weight Lifting Protocol:** Subjects were positioned in a seated position in a free-weight leg press machine with their legs raised to an angle of approximately 30° and weight was added using standard steel plates. Individuals performed 1-2 warm-up sets of 8-10 repetitions with minimal resistance to familiarize themselves with the machine. After becoming familiarized with the leg press machine, participants performed a warm up period of 1 to 2 sets of 10 repetitions at a perceived capacity of approximately 30 to 40% of their 1-repetition maximum (RM; calculated with a prediction equation$^4$). Then, 3 to 4 sets of 10 repetitions at a perceived capacity of approximately 80 to 90% of 1-RM were performed. A two minute rest interval was allotted between each set, and on the last repetition of the last set, subjects were instructed to hold the weight with their legs extended for 3 seconds by a member of the study team. Exercise heart rate and the 10-point Borg rating of perceived exertion (RPE) scale were used as indices of intensity after each set; however, all volunteers completed a minimum of 4 sets of 10 repetitions or to failure on the final set. Resting systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured manually after at least 10 minutes of rest using a standard mercury sphygmomanometer. Blood pressure was also measured manually during the last repetition of each set of exercise.

**References**